Scintigraphic Diagnosis of Syphilitic Lesions in Rabbits by Radiolabelled Monoclonal Antibodies Specific for *Treponema Pallidum*

MIN-GEOL LEE, KEE-YANG CHUNG, JONG DOO LEE, JEON SOO SHIN, KYU KWANG WHANG, OK DOO AHW, SE JONG KIM and JUNG BOCK LEE

Departments of Dermatology, Diagnostic Radiology and Microbiology, Yonsei University College of Medicine, Seoul, Korea, Department of Dermatology, Konkuk University College of Medicine, Chungju, Korea and Korea Atomic Energy Research Institute, Taejon, Korea

Immunoscintigraphy with radiolabelled monoclonal antibodies has been widely used to detect solid tumours. The purpose of this study was to investigate its potential for the specific localization of syphilitic lesions. F(ab′)2 fragments were prepared from murine monoclonal antibodies against *Treponema pallidum* produced in our laboratory and labelled with 111I. Bilateral testicular infections were created in rabbits by inoculation with *T. pallidum* or *Staphylococcus aureus*. Seven to 10 days after inoculation, radiolabelled antibodies were injected intravenously. Serial gamma images were then taken 2 h after the injection and at 24 h intervals thereafter. Beginning as early as 2 h post-injection, the testes could be visualized with either specific or non-specific antibodies. Gamma images in the monoclonal antibody-treated, *T. pallidum*-infected group persisted up to 48 h post-injection. Lesions were not discernible from background in the *S. aureus*-infected group injected with the monoclonal antibody and the *S. aureus*-infected and *T. pallidum*-infected groups injected with the polyclonal antibody at 24 h post-injection or later. Therefore, due to its ability to differentiate between specific and non-specific antibody-generated images from 24 h post-injection, immunoscintigraphy using monoclonal antibodies specific for *T. pallidum* may be employed as one of the methods to diagnose difficult cases of syphilitic internal organ involvement as well as syphilis infection in seronegative HIV-infected patients. Key word: Immunoscintigraphy.

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J. B. Lee, Department of Dermatology, Yonsei University College of Medicine, C.P.O. Box 8044, Seoul, Korea.

Since the report of tumour localization by radiolabelled antibodies directed against tumour antigens in 1953 (1), the in vivo delineation of tumour masses by the technique of “specific imaging” has received increasing attention (2). Polyclonal or monoclonal antibodies directed against tumour-associated or other antigens (3–5), when labelled with either radioiodine or indium-111 (111In), will localize in specific sites and can be detected by external scintigraphy with the conventional gamma cameras. In addition to imaging tumours or vascular diseases, the technique has recently been used for the specific localization of certain types of infections (6, 7). Rubin et al. (7) reported that radiolabelled murine monoclonal antibodies directed against Fisher immuneotype 1 *Pseudomonas aeruginosa* could be used to detect sites of infection with *P. aeruginosa*.

Although syphilis can usually be diagnosed with serologic tests for syphilis, these tests are not useful in detecting syphilitic internal organ involvement, from which it is difficult to detect *Treponema pallidum*. Thus a specific and non-invasive clinical test that can be performed to assess the presence and extent of syphilitic lesions is desirable.

To assess the potential of immunoscintigraphy for specific targeting of syphilitic lesions, we have utilized a rabbit model with testicular *T. pallidum* infection and have detected syphilitic lesions with a radiolabelled *T. pallidum*-specific monoclonal antibody.

**MATERIALS AND METHODS**

Microorganisms

*T. pallidum*, Nichols strain, from the Center for Disease Control, Atlanta, was used throughout the study. *T. pallidum* was purified from rabbit testicular tissue by Percoll density gradient centrifugation (7–10 days after inoculation into rabbit testicles) and 1 ml of 2 × 10^7* T. pallidum* was inoculated into both rabbit testes to induce an adequate inflammatory reaction. Negative control groups were injected with 1 ml of suspension containing 5 × 10^7 *S. aureus*. Immunoscintigraphy was performed 7–10 days after the inoculation, when the testicles were hardened and indurated by the inflammatory reaction. The *S. aureus* (ATCC 29213) was obtained from the American Type Culture Collection (Rockville, MD, U.S.A.).

**Rabbits**

Specific pathogen-free, 64 male New Zealand white rabbits weighing 2.5–3.0 kg were used for the experiments. They were housed for 2 weeks on standard diet before the experiments, and *T. pallidum* or *S. aureus* infection was produced in 32 rabbits each. Each group was subdivided into two groups, into which monoclonal antibody (MAb) and polyclonal antibody (PAb) were injected.

**Monoclonal antibody production**

MAb was made by immunizing BALB/c mice with *T. pallidum*. Their spleen cells were fused with SP2/0 or V93 myeloma cells and the clones secreting IgM, IgG and IgA antibodies were screened. Their isotypes were determined using a mouse monoclonal isotyping kit (HyClone Lab., Utah, U.S.A.). Among the MAbs produced, an antibody against the 47 kDa *T. pallidum* protein, YS-307 (IgG2a), was selected due to its high specificity and immunoreactivity as determined by ELISA. For the mass production of MAb, pristane (2, 6, 10, 14-tetramethyl pentadecane; Sigma Chemical Co., St. Louis, MO, U.S.A.) pretreated BALB/c mice were given YS-307 clone cells intraperitoneally. Ascitic fluid was collected 7–14 days later, centrifuged and the supernatant was collected (8).

**Monoclonal antibody purification**

A protein A-Sepharose CL-4B column, 1.0 cm x 10 cm, was used for the purification of MAb. Ascitic fluid was diluted with 15 ml of Tris buffer (pH 8.6) and passed through the column, and tubes showing an optical density value of more than 0.1 by ELISA were collected and concentrated to 1.5 mg/ml using Centriprep-10 (Amicon Division, Danvers, MA, U.S.A.) (8).

**Fragmentation of IgG to F(ab′)2**

IgG was digested into F(ab′)2, with pepsin at 37°C, pH 4.1 and pH 4.5. At the pepsin concentration of 0.1 mg/ml, incubation times of 2, 4, 6,
8, 12, and 16 h were given to determine the optimum condition. Digested products were purified by serial passage into the protein A-Sepharose CL-4B column and a Sephacryl-200 column (Pharmacia Fine Chemicals AB, Uppsala, Sweden) (9, 10).

Radioisotope labelling

Fab', fragments were labeled with 125I (Korea Atomic Energy Research Institute, Taejon, Korea) with chloramine-T. Approximately 60-70% of the 125I was labeled to yield a specific radioactivity of 1.2-3.5 mCi/mg of the antibody containing less than 3% of free iodide. Rabbit anti-human F(ab')2 specific for γ-chains (Dako Immunoc hemicals Inc., Copenhagen, Denmark) was labeled by the same method and used as PAb for control against YS-307.

Scintigraphy

Rabbits infected with T. pallidum or S. aureus were intravenously injected with 1 μCi of the radiolabeled monoclonal or polyvalent F(ab')2 fragments. Images were obtained by a standard field-of-view gamma camera (Siemens Gammascanies, Illinois, U.S.A.) at 2, 24 and 48 h after the injection. A high-energy parallel hole collimator with the energy level of 364 KeV was used. The images of each rabbit were obtained for a preset time of 300 s. The ratio of radioactivity of the lesion and the thigh area was calculated to obtain a target/background (T/B) radioactivity ratio. Radioactivities of the target (testes) and background (thigh muscles) were separately measured by sacrificing the rabbits 48 h after the inoculation.

Statistical analysis

Data obtained by scintigraphy were analyzed by the Mann-Whitney or Kruskal-Wallis tests, utilizing a SPSS/PC+ program installed in an IBM-compatible personal computer.

RESULTS

When MAb was used, testes infected with T. pallidum and S. aureus were clearly discernible from the surrounding tissue at 2 h post-injection. At 24 h, T. pallidum-infected testes were more prominent (Fig. 1), but the testes of the S. aureus-infected groups were not readily discernible from the surrounding tissue (Fig. 2). T. pallidum-infected testes still showed increased uptake at 48 h (Fig. 3). Both groups showed increased uptake at 2 h when polyvalent F(ab')2 was used, but...
diagnosing the disease as well as investigating the pathogenesis are needed. Serologic tests are the current mainstay for the diagnosis of syphilis, but internal organ involvement cannot be detected by the serologic methods alone — nor is it feasible to demonstrate the organism (14–16). Furthermore, in HIV-infected patients, serologic diagnosis may not be possible due to abnormal humoral immune response (17).

Immunoscintigraphy was first introduced to diagnose osteogenic sarcoma (1), using intact antibody to tumour cells. Only recently it has been used for the external detection of infectious diseases (6, 7). In contrast to the intact antibodies used in the previous studies, F(ab')2, or Fab fragments are more readily accumulated in the target tumours and excreted more rapidly from the blood and thus the background activity can be reduced. Non-specific accumulation in the liver can also be reduced (18–20). We have prepared F(ab')2 fragments from MAb specific for T. pallidum by pepsin digestion to eliminate the possibility of non-specific binding of the intact immunoglobulin to the Fc receptors on the neutrophils or macrophages in the infection foci. Pepsin digestion was also intended to reduce the background activity.

Rubin et al. (7) applied immunoscintigraphy for the external detection of Pseudomonas aeruginosa infection. When radiolabelled P. aeruginosa-specific MAb was injected, the images were clearly discernible from the group injected with radiolabelled non-specific antibody from 72 h after injection. In our experiments, the T. pallidum-infected group given YS-307 showed adequate contrast with the surrounding tissue from 2 to 48 h after injection. The group infected with S. aureus and given YS-307 and the groups infected with T. pallidum and S. aureus and given PAB showed adequate contrast with the surrounding tissue at 2 h, but the contrast was gone at 24 h. Therefore, the results between specific and non-specific antibodies were readily differentiated from 24 h after the injection. The reason for this discrepancy is probably due to the use of intact immunoglobulin by Rubin et al. (7). The excretion of intact immunoglobulin is prolonged due to its longer half-life as compared to F(ab')2, or Fab fragments (21).

**DISCUSSION**

Since the advent of antibiotics, the prevalence of syphilis throughout the world has decreased. VDRL-positive rate in Korea has also decreased from 2.5% in 1977 to 0.4% in 1990 (11). However, partly due to the widespread use of drugs and prostitution, syphilis prevalence in the United States has markedly increased since 1986 (12, 13). Owing to the recent world-wide increase in the number of drug users and the spread of HIV, syphilis may once again become a global health problem.

To effectively handle the disease, more effective means of
We consider that F(ab')2, fragments of the YS-307, the MAb against T. pallidum, can be utilized for immunoscintigraphy to detect internal syphilitic involvement, although further evaluation in human syphilis patients matched with actual demonstration of T. pallidum is required for diagnostic as well as investigative purposes.

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REFERENCES